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MORPHOLOGICALLY CRYPTIC SPECIES WITHIN *DOWNINGIA YINA* (CAMPANULACEAE)

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ABSTRACT

The *Downingia yina* species complex (Campanulaceae), centered in northern California and southern Oregon, currently contains three morphologically distinguished species: *D. yina*, *D. elegans*, and *D. bacigalupii*. This complex of species is notable for high levels of morphological and cytological variation, with chromosome counts of $n = 6$, 8, 10, and 12. Molecular evidence suggests three main clades within this complex, corresponding more with cytological variation than with morphological variation. Additionally, the molecular evidence suggests a phylogeographic pattern associated with the Cascade Ranges, where members of the clade characterized by chromosome counts of $n = 6$, 8, and 10 are distributed primarily to the west of the Cascades while members of the clade characterized by chromosome counts of $n = 12$ are distributed primarily to the east. A third clade characterized by $n = 10$ is localized in the Lake of the Woods region of southern Oregon. Evidence from morphological, cytological, interfertility, and molecular data was used to re-examine the delimitation of species within this complex. *Downingia elegans* and *D. bacigalupii* are maintained, while *D. yina* is split into three morphologically cryptic species (*D. yina*, *D. willamettensis*, *D. pulcherrima*) that do not form a clade.

Key Words: Campanulaceae, chromosome races, cryptic species, *Downingia*, phylogeography.

The *Downingia yina* species complex is a monophyletic group (Schultheis 2001) comprising *D. yina* Applegate, *D. bacigalupii* Weiler, and *D. elegans* (Lindl.) Torr. The species complex represents a cytologically and morphologically variable group centered in northern California and southern Oregon. Chromosome numbers within the complex include $n = 12$ in *D. bacigalupii*, $n = 10$ in *D. elegans*, and races of $n = 6$, 8, 10 and 12 in *D. yina* (Weiler 1962; Foster 1972; Lammers 1993). Morphologically, both *D. bacigalupii* and *D. elegans* are distinguished from *D. yina* by an exserted staminal column with a sharp bend between the anthers and filament, and by the concave oval-shaped lower corolla lip with relatively parallel corolla lobes. *Downingia bacigalupii* can be distinguished from *D. elegans* by the corolla's lighter shade of purple and by the yellow pigmentation in the corolla throat, a feature also found in *D. yina*.

Morphological variation within *D. yina* has led some workers to recognize additional species or infraspecific taxa. *Downingia yina* was described by Applegate (1929) from a localized region of the southern Cascade Ranges in Klamath Co., Oregon. Shortly thereafter, Peck (1934, 1937) described two additional larger flowered species: *D. willamettensis* Peck from the Willamette Valley of Oregon, and *D. pulcherrima* Peck from eastern Oregon. In the first monograph of the genus, McVaugh (1941) recognized *D. yina* and *D. willamettensis*, including *D. pulcherrima* in the latter. McVaugh noted (1941), however, that *D. yina* and *D. willamettensis* were not readily distinguishable, and ultimately treated them as varieties within *D. yina*, var. *yina* and var. *major* McVaugh, respectively (McVaugh 1943). He distinguished the two varieties based on fruit characteristics (fusiform with hyaline lines in var. *yina*; subulate without hyaline lines in var. *major*), plant stature (larger and more erect in var. *major*), and geographic location of the populations. Weiler (1962) found that the differences described between *D. yina* and *D. willamettensis* were not maintained under greenhouse conditions. He accordingly recognized only *D. yina* with no infraspecific taxa, although noting that fresh material of *D. pulcherrima* was not examined. Weiler (1962) also noted that individuals of *D. yina sensu lato* tended to be decumbent to the west of the Cascade Ranges, and erect to the east. Foster (1972) was unable to find consistent morphological differences to correspond with cytological races in *D. yina*, but did note an ecological trend. She observed that *D. yina* chromosome races $n = 6$, 8, and 10 are found in habitats characterized by Küchler (1964) as Oregon-oak woodland or cedar-hemlock-Douglas fir mosaic while the *D. yina* chromosome race $n = 12$ is found in California mixed evergreen forest and juniper-steppe woodland, as characterized by Küchler (1964). Both Foster (1972) and Ayers (1993) followed Weiler (1962) in recognizing only *D. yina*.

The present study emerged largely from a systematic investigation of the genus *Downingia*, in which molecular data unexpectedly suggested the existence of morphologically cryptic lineages within *D. yina* (Schultheis 2001), corresponding in part to infraspecific taxa previously recog-

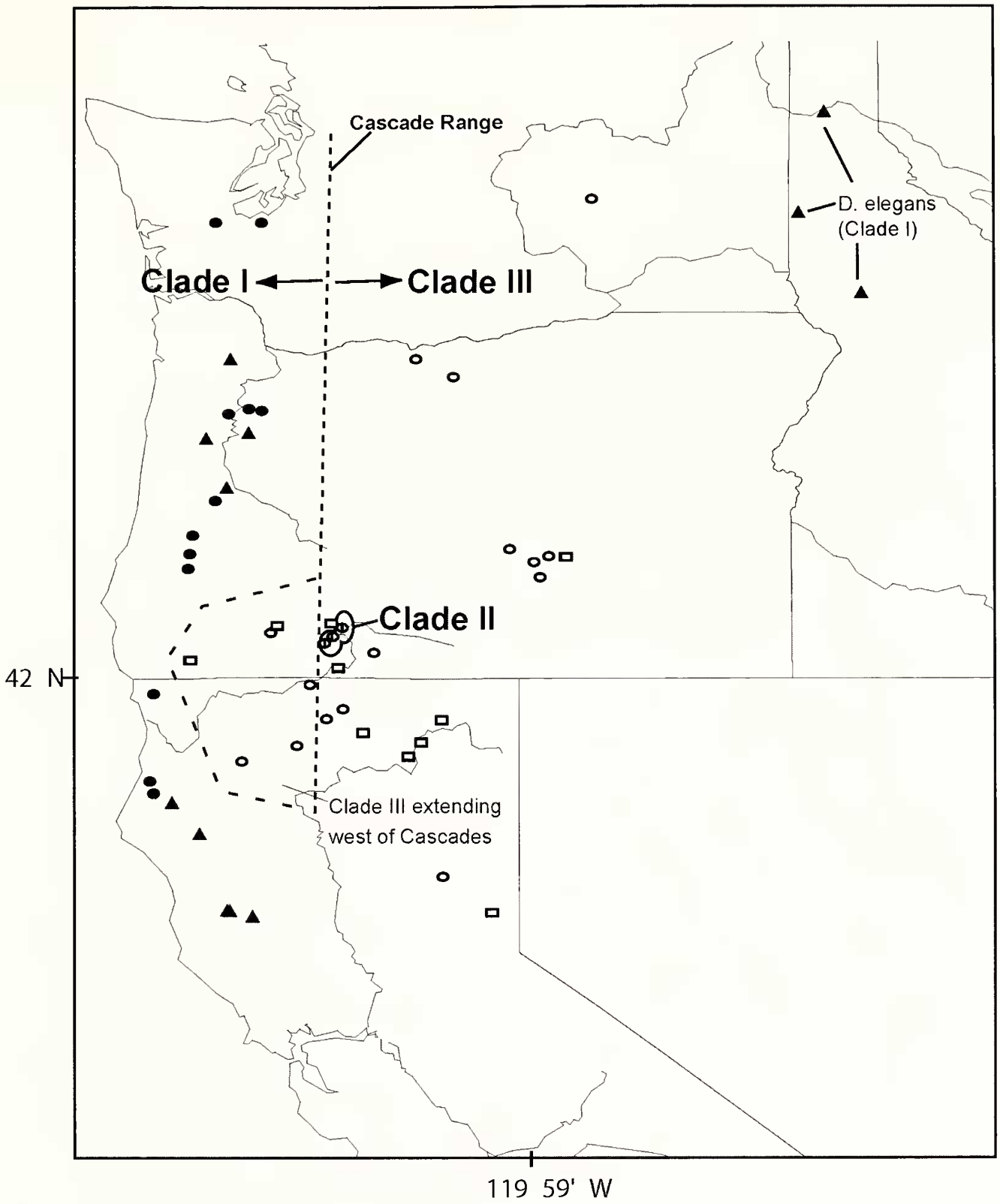

FIG. 1. Map of the northwestern USA showing localities of samples included in this study. Each symbol may represent one or multiple samples from the vicinity. The dashed line roughly corresponds to the geographic barrier created by the Cascade Range. Triangles = *Downingia elegans*. Squares = *D. bacigalupii*. Circles = samples previously included in *D. yina*. Filled circles = now assigned to *D. willamettensis*. Open circles = now assigned to *D. pulcherrima*. Circles with line through center = now assigned to *D. yina sensu strictu*. Clade I, Clade II and Clade III refer to clades identified in phylogenetic analyses.

nized. The situation was further complicated (Schultheis 2001) by the apparent para- or polyphyly of *D. yina* with respect to *D. elegans* and *D. bacigalupii*. The *D. yina* species complex thus represents a mixture of morphologically cryptic and morphologically distinctive lineages that may not correspond to the species currently recognized (Ayers 1993). The aim of this study was to further investigate the relationships and circumscriptions of *D. bacigalupii*, *D. elegans* and *D. yina* using morphological data, additional nuclear and chloroplast molecular sequence data to supplement Schultheis (2001), and available cytological and interfertility data (Weiler 1962; Foster 1972).

METHODS

Taxon Sampling

Collections were made from throughout the range of *D. elegans*, *D. bacigalupii*, and *D. yina* (Appendix 1; Fig. 1). Herbarium collections provided important supplemental material. *Downingia bicornuta* A.Gray, *D. concolor* E. Greene, *D. cuspidata* (E. Greene) Rattan, *D.*

TABLE 1. CHARACTERS USED IN MORPHOLOGICAL ANALYSES OF THE *DOWINGIA YINA* COMPLEX. Characters 1–10 are quantitative and measured in millimeters, characters 11–14 are qualitative, and characters 15–19 are ratios. [1] Characters used in cladistic analyses, with character states noted in brackets.

Character number	Character	Character definition and how assessed
1.	sepal	dorsal sepal, length
2.	back slit	corolla base to dorsal slit, length; equivalent to height of corolla tube along dorsal surface
3.	side slit	corolla base to lateral slit, length; equivalent to height of corolla tube along lateral surface
4.	upper lobe	upper corolla lobes, length
5.	lower lobe	lower corolla lip, length
6.	filament[1]	filament tube, length (<6 mm [0], >6 mm [1])
7.	anther	anther tube, length
8.	anther angle[1]	angle between anther and filament tubes (<50 [0], >70 [1])
9.	lower angle	angle of divergence between lobes of the lower corolla lip
10.	horns[1]	anther horns, length (<0.62 mm [0], >0.62 mm [1]); refers to triangular projections on each of the two smaller anthers
11.	anther back	trichomes on anther dorsal surface: abundant (0), few (1), none (2)
12.	upper lobe orientation	upper corolla lobes, orientation: parallel (0), intermediate (1), divergent (2)
13.	yellow[1]	yellow on lower corolla lobe: present (0), absent (1)
14.	lower lobe shape	lower corolla lobe, shape: acute (0), intermediate (1), mucronate (2)
15.	filament/anther	filament length/Anther length
16.	sideslit/backslit[1]	length of lateral slit/Length of dorsal slit (≥0.6 [0], <0.6 [1])
17.	filament/backslit[1]	filament length/Length of dorsal slit (≤1 [0], 1–2 [1], >2 [2])
18.	upper/lower lobe	upper lobes length/Lower lip length
19.	backslit/upper lobe	length of dorsal slit/Upper lobes length

montana (E. Greene) Rattan, and *D. ornatissima* E. Greene were chosen as outgroup taxa based on previous phylogenetic analyses within the genus (Schultheis 2001).

Molecular

Generation of sequence data. Extraction of total DNA from 24 samples first reported in Schultheis (2001) and 9 new samples (Appendix 1) involved use of either the CTAB protocol of Doyle and Doyle (1987) or Hillis et al. (1996) with minor modifications (Schultheis 2001), or use of Qiagen DNeasy Plant mini kits following manufacturer's instructions. Most plant tissue samples were stored in a cooler while in the field and transferred to a −80C freezer within one week of collection. Voucher specimens were either the same plant from which tissue for DNA extraction was taken, or were other plants from the same site.

Sequence data were generated from the nuclear 18S–26S rDNA internal transcribed spacer (ITS) and the chloroplast 3′*trn*K intron. Amplification and sequencing methods changed during the course of the project, as new techniques became available. Single-stranded DNAs of ITS 1 and ITS 2 were generated, purified, and manually sequenced following Baldwin (1992). Double-stranded DNAs of ITS 1, ITS 2 and the 3′*trn*K intron were generated, purified, and sequenced using automated sequencing technology following Schultheis (2001). Sequences are deposited in Genbank.

Sequence alignment. All alignments were visual. Sites coded with "?" or with an IUPAC-IUB ambiguity code represent basepairs where sequence produced with neither primer produced a sufficiently strong or clear signal for confident basepair assignment. Indels, coded as "-", were treated as missing data. Two regions were excluded from the ITS dataset due to ambiguous sequence alignment (positions 132–138 and 284–291 of the aligned ITS data set).

Evaluation of sequence data. Separate and combined analyses using a parsimony criterion were conducted for ITS and 3′*trn*K intron data. All analyses employed heuristic searches with 10,000 replicates of random sequence addition and tree-bisection-reconnection (TBR) branch swapping. Conservative estimates of clade support were assessed using 10,000 replicates of the "fast" bootstrap option in PAUP 4.0b5. Decay analyses (Donoghue et al. 1992; Bremer 1994) using Autodecay (Eriksson 1998) were conducted for the 3′*trn*K intron and the combined molecular analyses.

Morphology

Fresh and/or herbarium material was examined from 80 localities (Appendix 1, Fig. 1) and 450 flowers. Nineteen characters were included for phenetic analyses, including 10 quantitative, 4 qualitative, and 5 ratio characters (Table 1). All

characters are floral, because vegetative characters are not generally useful for distinguishing species of *Downingia*. Characters were observed or measured against a ruler under a dissecting scope, except for anther horn length which was measured with an ocular micrometer.

Morphometric analyses. Analyses of variance were conducted to identify characters differing significantly among the three currently recognized species, and Tukey tests were used to identify which species differed. The same was done within *D. yina* for the three groups identified by molecular analyses (see results). For multivariate analyses, a data matrix was created containing the average value for each character from each collection locality. Multivariate analyses included cluster analysis using Euclidean distances and single linkage, discriminant function analysis, and Principal Components Analysis (PCA), the latter using a matrix standardized so that each character had a mean of zero and a standard deviation of one. All statistical analyses were performed with SYSTAT 5.2.1.

Cladistic analyses. One qualitative and five quantitative characters (indicated in Table 1) were used in a cladistic analysis of the 26 populations for which molecular data were also available, plus one population per outgroup taxon. Phylogenetic analyses using a parsimony criterion were conducted with PAUP 3.1.1 (Swofford 1993) or PAUP *4.0b5. The analysis employed a heuristic search with 100 replicates of random taxon addition and TBR branch-swapping. Qualitative characters excluded from the analysis were polymorphic within most populations. Character states for the quantitative characters were determined by searching for gaps within the character distribution among specimens that were greater than 2 times the average population standard deviation (Archie 1985). Most quantitative characters were excluded from the cladistic analysis because no character states could be defined. The character "locule", referring to the number of locules in the ovary, separates the *D. yina* complex from the outgroup taxa. The morphological data matrix is provided in Table 2.

Cytology

Chromosome counts were obtained from unpublished theses (Weiler 1962; Foster 1972) and from numerous specimens deposited at the UC and JEPS herbaria as chromosome vouchers (Appendix 1). Chromosome number was treated as an ordered character. All known chromosome counts for *D. bacigalupii*, *D. elegans*, *D. bicornuta*, *D. cuspidata*, *D. ornatissima* and *D. montana* report a single number for each of the species (Wood 1961; Foster 1972; Weiler 1962; Lammers 1993). All samples of these taxa were scored based on chromosome counts reported for the species, regardless of whether a count was obtained from the population sampled here. The only exception is *D. bacigalupii* sample 585-99, which was scored as unknown since the population is at the limits of the species range, and no chromosome counts were available from the vicinity. Chromosome counts for *D. concolor* are $n = 8$ and $n = 9$ (Weiler 1962; Lammers 1993). The samples of *D. concolor* included here fall within the known geographic range of $n = 9$ reports for *D. concolor* (Weiler 1962), and were scored as such. Populations of *D. yina* were scored based on the geographic proximity of the population to a population with a documented chromosome number (indicated in Table 2; Weiler 1962; Foster 1972). MacClade version 3.0 (Maddison and Maddison 1992) was used to reconstruct the most parsimonious chromosome numbers characterizing each node on trees produced from the combined analysis of the ITS and 3′*trn*K datasets.

Analyses of Combined Molecular, Morphological, and Cytological Data

A partition-homogeneity test (Farris et al. 1995) performed in PAUP *4.0 (Swofford 2001) confirmed combinability of the ITS plus 3′*trn*K data ($P = 0.247$; 1000 replicates, heuristic searches with random addition and TBR branch swapping), and of the molecular data with the morphological and cytological data ($P = 0.094$). Morphological and cytological data were combined as a single partition for the test since cytological data consisted of only one character (chromosome number). A branch and bound search of the combined data was conducted under a parsimony criterion. Clade support was assessed using 10,000 replicates of the "fast" bootstrap option. The morphological data came from the same or neighboring populations as the sequence data (Table 2). The cytological data consisted of chromosome numbers and did not include information regarding meiotic configurations of chromosomes in hybrid plants.

Interfertility

Information regarding interfertility and crossability among members of the *D. yina* complex comes from Weiler's unpublished thesis (1962), in which he documented the results of numerous interspecific crosses within *Downingia*. His data include qualitative assessments of seed set, germination, and hybrid condition (e.g., flowering, green, chlorotic, dying in seedling stage), some quantitative assessments of pollen stainability, and analysis of meiotic configurations.

Information regarding interfertility and crossability within *D. yina* comes from Foster's

TABLE 2. MORPHOLOGICAL DATA MATRIX USED FOR CLADISTIC ANALYSES. Sample numbers correspond to Appendix 1, with the following prefixes: B = *Downingia bacigalupii*, E = *D. elegans*, Y = *D. yina*, M = *D. montana*, C = *D. concolor*, O = *D. ornatissima*, BI = *D. bicornuta*, CU = *D. cuspidata*. Characters and states are listed in Table 1. The "locule" character refers to the number of locules in the ovary [bilocular (0), unilocular (1)]. For *D. yina* the "chromosome" character refers to the chromosome number based on reports or vouchers from the same or a neighboring population, indicated in parentheses. This character was included in the analyses of all data combined, but was not included in the analysis of morphological data alone. For some samples, the morphological data were combined with the molecular data from a neighboring population, indicated in parentheses.

Sample	Character							
	6	8	10	13	16	17	Locule	Chromosome
B *Schultheis 585-99*	0	1	1/0	0	0	1	1	?
B *Schultheis 240-95*	1	1/0	1/0	0	0	1	1	12
B *Schultheis 237-95*	1	1	0	0	0	1/2	1	12
B *Schultheis 231-95*	1	1	1/0	0	0	1	1	12
B *Schultheis 251-95*	1	1	0	0	1/0	1/2	1	12
E *Schultheis 243-95*	1	1	0	1	1	1	1	10
E *Schultheis 242-95*	0	1	0	1	1	1/0	1	10
E *Schultheis 320-96*	0	1	0	1	1/0	1	1	10
E *Weiler 60138* (Foster 70-15-4)	0	1/0	0	1	0	0	1	10
Y *Oswald & Ahart 3943*	0	0	0	0	1/0	1	1	?
Y *Schultheis 247-95*	0	0	0	0	0	0	1	10 (Foster 70-96-11)
Y *Tracy 3217*	0	0	0	0	0	0	1	10 (Foster 70-96-11)
Y. *T. Obrien s.n.*	0	0	0	0	0	0	1	?
Y *Schultheis 236-95*	0	0	0	0	0	0	1	12 (Weiler 60207)
Y *D. Barbe 348*	0	0	0	0	0	1/0	1	12 (Foster, Siskiyou)
Y *Schultheis 241-95*	0	0	1/0	0	0	0	1	12 (Foster, Harney)
Y *Schultheis 584-99*	0	0	0	0	0	0	1	12 (Foster 70-43-15)
Y *Schultheis 245-95*	0	0	0	0	0	1/0	1	12 (Foster 70-43-15)
Y *Weiler 61449*	0	0	0	0	0	0	1	10 (Weiler 61200)
Y *Schultheis 581-99*	0	0	0	0	0	0	1	10 (Weiler 61451)
Y *R. Bacigalupi 7978*	0	0	0	0	0	0	1	10 (Weiler 61200)
Y *Cook 962*	0	0	0	0	0	0	1	8
Y *Peck 16291* (Foster 68-210)	0	0	0	0	0	0	1	6 (Foster 68-210)
Y *Schultheis 319-95*	0	0	0	0	1/0	0	1	12 (Weiler 61383)
Y *Weiler 61333* (Foster 68-51)	0	0	0	0	0	0	1	10
Y *R. Bacigalupi 7894*	0	0	0	0	0	0	1	12 (Foster,Wasco)
BI *Schultheis 100-95*	0	0	1/0	0	0	0	0	11
C *Schultheis 195-95*	0	0	0	0	0	1/0	0	9
M *Schultheis 235-95*	0	0	0	0	0	0	1	11
CU *Schultheis 179-95* (197-95)	0	0	0	0	0	0	0	11
O *Schultheis 180-95*	0	0	0	0	0	1	0	12

unpublished thesis (1972). She documents meiotic configurations and pollen stainability for crosses between individuals of the same and different chromosome races. I assigned each of Foster's parent populations to a molecular clade, based either on sequence data from her voucher specimens, or on close proximity of the vouchered population to a population with sequence data. I applied an ANOVA to Foster's raw pollen stainability data to examine whether there were significant decreases in stainability in hybrids between versus within chromosome races, and between versus within molecular clades.

RESULTS

Cladistic Analyses

Levels of divergence for the ITS dataset ranged from 0.0 to 0.017, excluding outgroups. Analysis of ITS data resulted in 104 minimum-length trees based on 43 parsimony-informative characters (length = 100; CI = 0.90, 0.83 without uninformative characters; RI = 0.91). Levels of divergence for the 3′*trn*K dataset ranged from 0.0 to 0.027, excluding outgroups. Analysis of the 3′*trn*K dataset resulted in 42 trees based on 19 parsimony-informative characters (length = 67; CI = 0.94, 0.83 without uninformative characters; RI = 0.89). Combined molecular analyses produced 2 trees based on 43 parsimony-informative characters (length = 158; CI = 0.91, 0.77 without uninformative characters; RI = 0.89). Combined molecular, morphological and cytological analyses produced 72 trees based on 50 parsimony-informative characters (length = 176; C.I. = 0.87, 0.72 without uninformative characters; RI = 0.86).

All analyses (ITS dataset; 3′*trn*K dataset; combined molecular datasets; combined molecu-

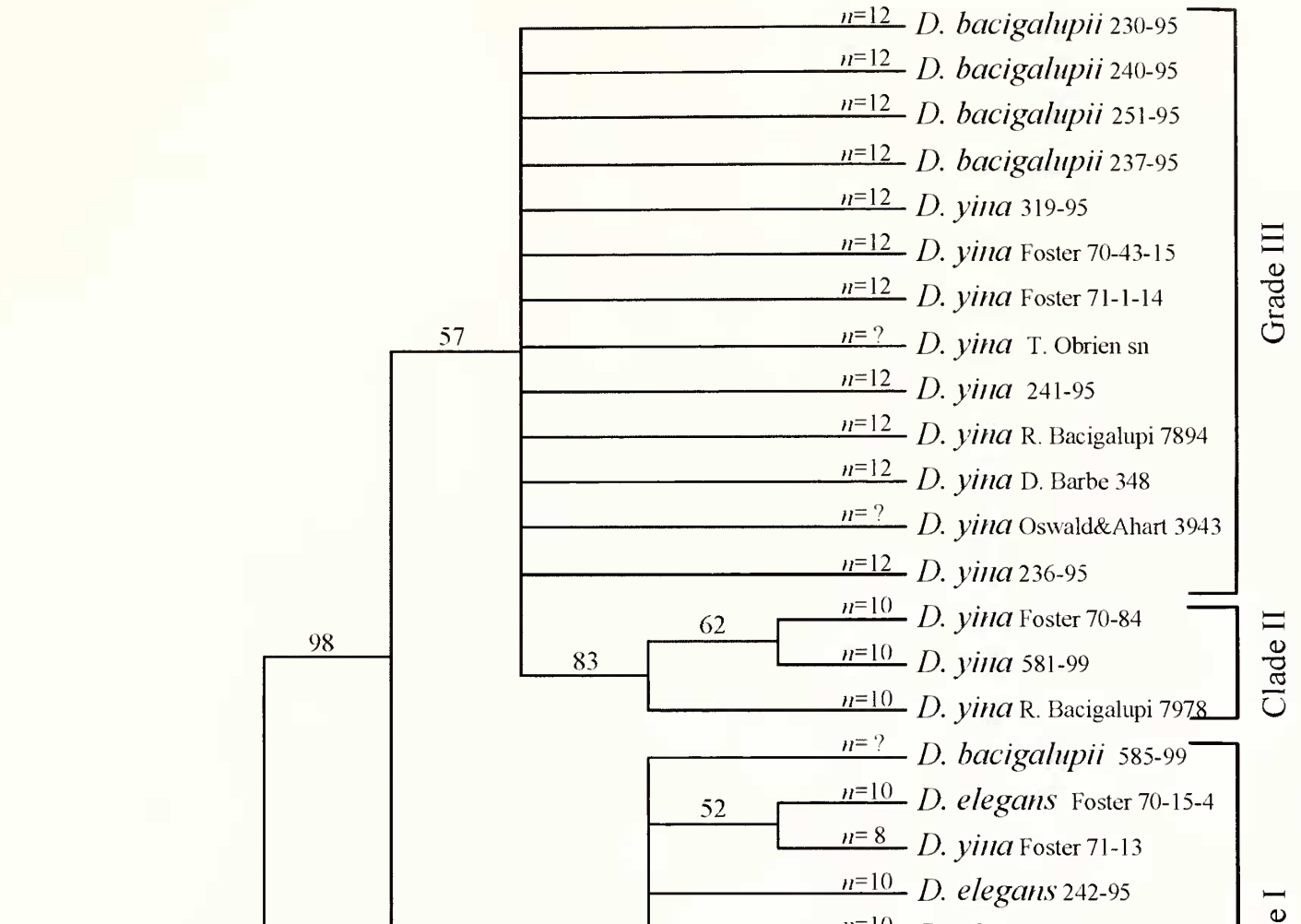

FIG. 2. The strict consensus of 104 minimum-length ITS parsimony trees (length = 100; C.I. = 0.90, 0.83 w/o uninformative characters; R.I. = 0.91) produced from a heuristic search with 10,000 replicates of random taxon addition and TBR branch swapping. Numbers above the branches indicate bootstrap values generated from 10,000 replicates of the "fast" bootstrap method. Sample collection numbers correspond to Appendix 1. If only a number is indicated, the collection was by Schultheis. Chromosome counts are uniform for all species except *Downingia yina*. Sources for *D. yina* chromosome counts are indicated in Table 2.

lar, morphological and cytological datasets) except that of morphological data alone resulted in three main clades or grades (Figs. 2–5). Clade I comprised *D. elegans* and *D. yina* pro parte. Clade II comprised *D. yina* pro parte. Clade III comprised *D. bacigalupii* and *D. yina* pro parte. Primary differences among the trees produced from different analyses were the following: (1) There was a sister relationship between Clades I and II in trees resulting from analyses of all datasets but the ITS dataset, in which Clade II is aligned with grade III (Fig. 2). (2) *Downingia elegans* sample Foster 70-15-4 was resolved as part of Clade I in all trees except those resulting from analysis of the 3′*trn*K dataset, in which it fell in an unresolved position between Clades I and II (Fig. 3). This sample is from Snow Mountain, in Lake Co., California, at the southern limit of the species range (Fig. 1). (3) *Downingia bacigalupii* sample 585-99 is aligned with Clade I in ITS trees (Fig. 2), but is sister to other members of Clade III in all other trees. Sample 585-99 is from Josephine Co., Oregon, at the western periphery of the species range

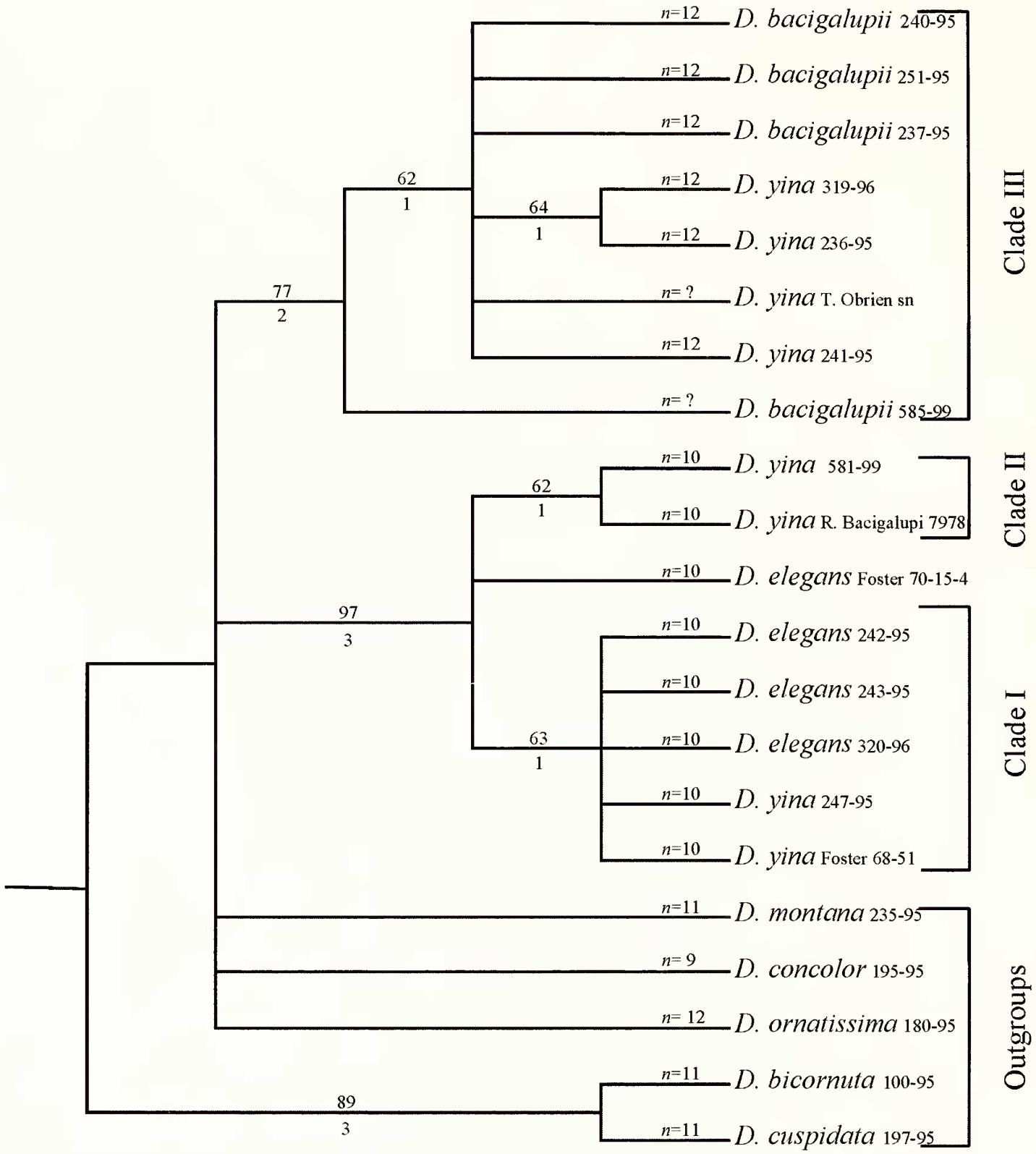

FIG. 3. The strict consensus of 42 minimum-length 3′*trn*K intron trees (length = 67; C.I. = 0.94, 0.83 w/o uninformative characters; R.I. = 0.89) produced from a heuristic search with 10,000 replicates of random taxon addition and TBR branch swapping. Numbers above the branches indicate bootstrap values generated from 10,000 replicates of the "fast" bootstrap method. Numbers below the branches are decay indices. Sample collection numbers correspond to Appendix 1. If only a number is indicated, the collection was by Schultheis. Chromosome counts are uniform for all species except *Downingia yina*. Sources for *D. yina* chromosome counts are indicated in Table 2.

(Fig. 1). (4) When morphological and cytological data are combined with molecular data, the resulting trees resolve samples of *D. elegans* and of *D. bacigalupii* as clades within Clade I and Clade III respectively (Fig. 5). *Downingia bacigalupii* sample 585-99, however, is resolved as sister to Clade III, and *D. elegans* sample Foster 70-15-4 is unresolved within Clade I.

The strict consensus of 2556 trees based on six parsimony-informative characters (length = 9, C.I. = 0.67, RI = 0.88) produced by the cladistic analysis of only the morphological data showed no resolution (figure not shown).

Morphometric Analyses

Downingia yina complex. Univariate analyses within the *D. yina* complex showed that the three currently recognized species were significantly different from one another for numerous characters, although ranges overlapped for all characters (Table 3). Anther angle and the angle of

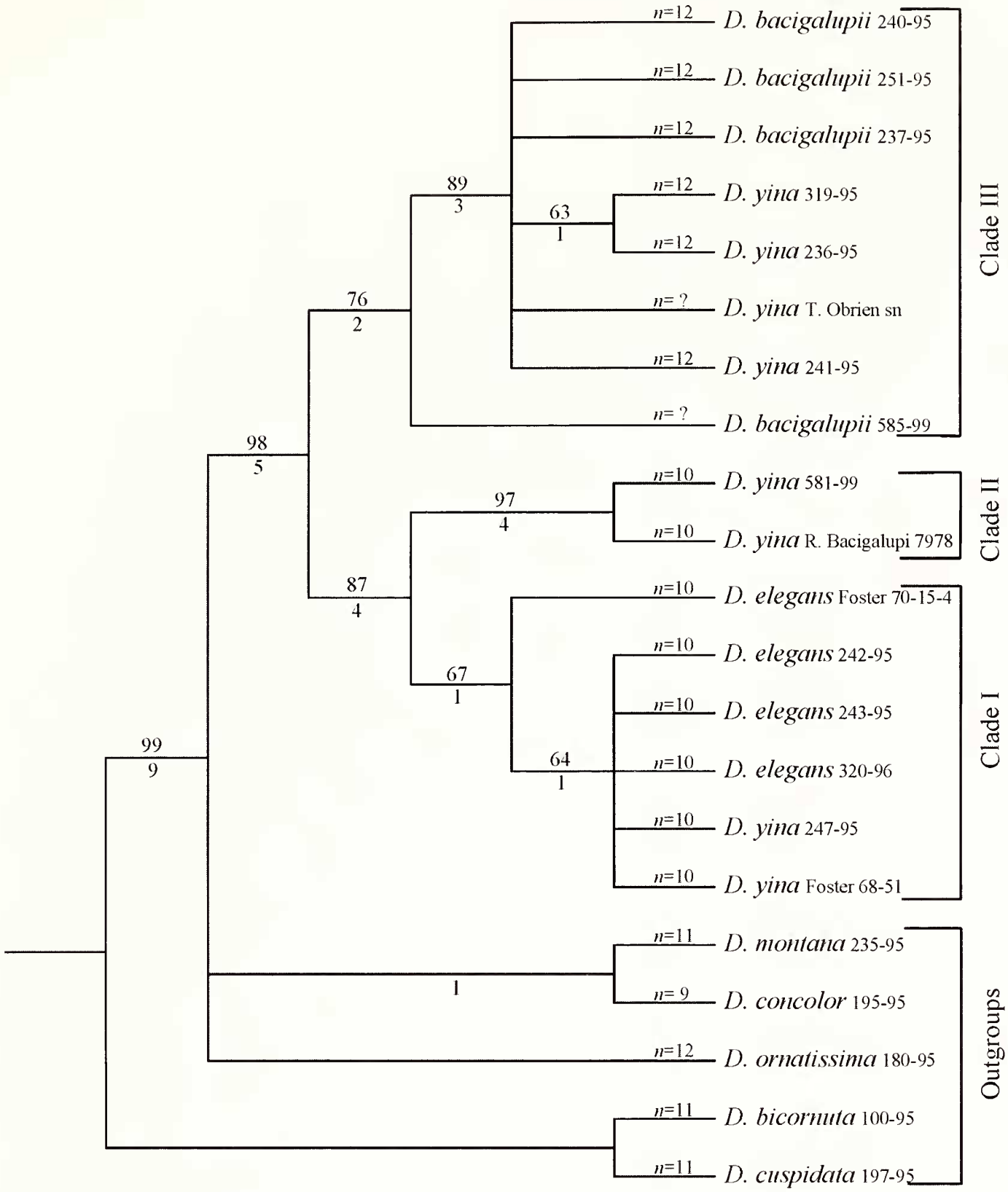

FIG. 4. The strict consensus of two minimum-length trees (length = 158; C.I. = 0.91, 0.77 w/o uninformative characters; R.I. = 0.89) from a heuristic search of combined ITS and 3′*trn*K intron data, with 10,000 replicates of random taxon addition and TBR branch swapping. Numbers above the branches indicate bootstrap values generated from 10,000 replicates of the "fast" bootstrap method. Numbers below the branches are decay indices. Sample collection numbers correspond to Appendix 1. If only a number is indicated, the collection was by Schultheis. Chromosome counts are uniform for all species except *Downingia yina*. Sources for *D. yina* chromosome counts are indicated in Table 2.

divergence between lobes of the lower corolla lip in particular distinguished *D. elegans* and *D. bacigalupii* from *D. yina*. The former two taxa had more sharply bent anthers and less divergent lower corolla lobes than *D. yina*. The filament of *D. bacigalupii* was longer on average than that of *D. elegans* and *D. yina*. Additionally, *D. elegans* could be distinguished by the lack of yellow pigmentation on the lower corolla lip.

PCA analyses of all samples using all data showed clear separation among *D. elegans*, *D. bacigalupii*, and *D. yina*, particularly when principal components I and III were plotted (Fig. 6). This separation was also clear when only ratio characters were used or when ratio characters were excluded. Characters of particular importance in the PCA analyses were the anther angle, the filament/back slit ratio, and the angle of divergence between the lower corolla lobes. The percent of total variance explained by components I, II, and III was 45.2%, 17.5%, and 12.0%, respectively.

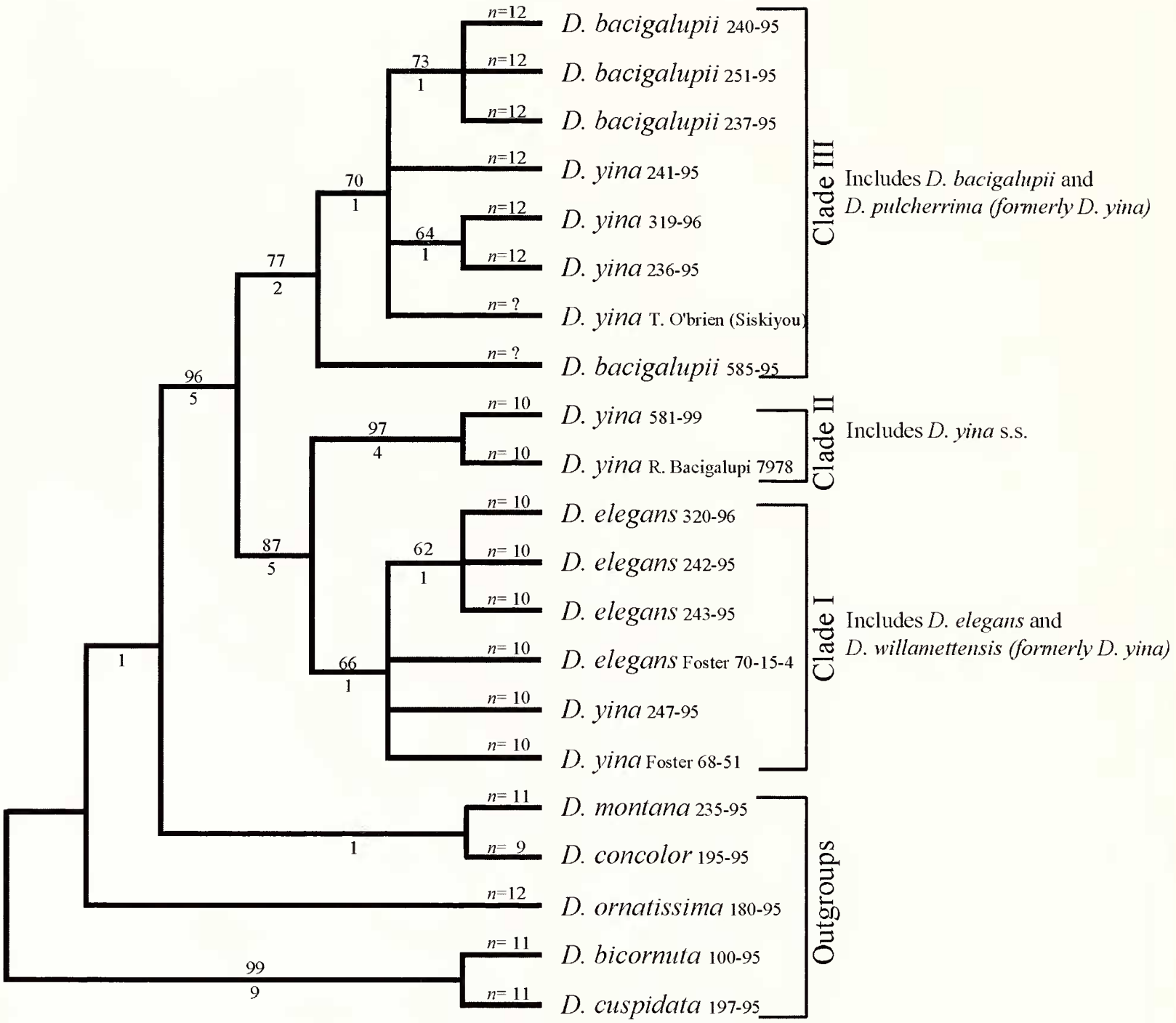

FIG. 5. The strict consensus of 72 minimum-length trees (length = 176; C.I. = 0.87, 0.72 w/o uninformative characters; R.I. = 0.86) produced from a branch-and-bound search of combined data. The data matrix included the ITS, 3′*trn*K, morphological, and cytological data. Numbers above the branches indicate bootstrap values generated from 10,000 replicates of the "fast" bootstrap method. Numbers below the branches are decay indices. Sample collection numbers correspond to Appendix 1. If only a number is indicated, the collection was by Schultheis. Chromosome counts are uniform for all species except *Downingia yina*. Sources for *D. yina* chromosome counts are indicated in Table 2.

Cluster analysis (not shown) produced two main groups, one with *D. yina* samples and the other with a mixture of *D. elegans* and *D. bacigalupii* samples. One sample of *D. elegans* (*Ehlers & Erlanson 39*) and one sample of *D. bacigalupii* (582-99) together joined at the base of the *D. yina* cluster.

Variation within Downingia yina. Univariate analyses revealed that significant character differences were evident between *D. yina* samples from the three main molecular clades, but with overlapping ranges (Table 4). No qualitative characters could be used to uniquely identify the three groups. In general, Clade II samples tended to be smaller for most quantitative characters measured (Table 4). Samples from Clade I tended to have a less sharply bent anther and a wider angle of divergence between the lobes of the lower corolla lip than did samples from Clades II and III.

PCA analyses of only *D. yina* samples using all data did not clearly distinguish between samples from Clades I, II, and III (not shown). Discriminant function analysis of *D. yina* samples showed better separation of the three groups, but with areas of overlap (Fig. 7). Characters of particular importance in the discriminant function analyses were anther length, the angle of divergence between the lobes of the lower corolla lip, and trichome density on the dorsal anther surface.

Cluster analysis (not shown) grouped all of the *D. yina* samples together, but did not resolve groups corresponding to Clade I, II, and III samples.

TABLE 3. UNIVARIATE STATISTICS FOR THE *DOWNINGIA YINA* COMPLEX. Means, standard deviations and ranges (in parentheses) are provided for each character within each species. Superscripts indicate groups that are significantly different from one another using Tukey multiple comparison tests following ANOVA. Groups with no superscript or that share a superscript are not significantly different.

Character	*D. elegans* (n = 62)	*D. yina* (n = 317)	*D. bacigalupii* (n = 69)
Sepal (mm)	5.31 ± 1.30^{A} (3.0–8.0)	4.75 ± 1.28^{B} (0.50–10.0)	5.81 ± 1.80^{A} (3.0–10.0)
Side slit (mm)	2.33 ± 0.45^{A} (1.5–3.0)	4.10 ± 0.71^{B} (1.75–6.0)	2.96 ± 0.54^{C} (2.0–4.25)
Back slit (mm)	4.48 ± 0.75^{A} (3.0–6.0)	4.80 ± 0.89^{B} (2.25–7.5)	3.87 ± 0.91^{C} (2.25–6.0)
Upper lobe (mm)	4.04 ± 1.16^{A} (2.0–7.0)	3.71 ± 0.93^{A} (2.0–6.75)	6.75 ± 1.35^{B} (4.0–11.0)
Lower lobe (mm)	6.53 ± 1.82^{A} (3.5–12.0)	6.18 ± 1.30^{A} (3.0–9.5)	8.40 ± 1.80^{B} (5.0–14.0)
Filament (mm)	5.27 ± 1.48^{A} (2.5–7.5)	3.17 ± 0.92^{B} (1.25–5.75)	7.48 ± 1.50^{C} (3.25–9.75)
Anther (mm)	2.68 ± 0.46^{A} (1.5–3.5)	2.18 ± 0.36^{B} (1.25–3.0)	2.89 ± 0.42^{C} (1.25–3.75)
Anther angle (degrees)	84.07 ± 14.40^{A} (28.0–90.0)	22.18 ± 9.11^{B} (0.0–51.0)	88.80 ± 6.75^{A} (38.0–90.0)
Lower angle (degrees)	9.16 ± 11.92^{A} (0.0–50.0)	53.69 ± 12.79^{B} (20.0–90.0)	12.31 ± 9.88^{A} (0.0–30.0)
Horns (mm)	0.44 ± 0.08^{A} (0.26–0.75)	0.41 ± 0.11^{A} (0.13–0.79)	0.60 ± 0.10^{B} (0.32–0.86)
Filament/anther	1.95 ± 0.34^{A} (1.2–2.55)	1.45 ± 0.30^{B} (0.625–2.375)	2.58 ± 0.35^{C} (1.6–3.6)
Side slit/back slit	0.53 ± 0.10^{A} (0.35–1.0)	0.86 ± 0.09^{B} (0.47–1.3)	0.79 ± 0.13^{C} (0.5–1.11)
Filament/back slit	1.18 ± 0.23^{A} (0.56–1.75)	0.66 ± 0.18^{B} (0.38–2.0)	1.97 ± 0.36^{C} (1.42–3.56)
Upper lobe/lower lobe	0.64 ± 0.15^{A} (0.25–1.0)	0.61 ± 0.14^{A} (0.31–1.28)	0.81 ± 0.14^{B} (0.54–1.14)
Back slit/upper lobe	1.19 ± 0.35^{A} (0.6–2.22)	1.37 ± 0.41^{B} (0.5–3.11)	0.59 ± 0.18^{C} (0.35–1.14)

Cytology

Chromosome numbers within the *Downingia yina* complex appear to correspond to the molecular clades identified with ITS and 3′*trn*K sequences (Figs. 2–4, Appendix 1). All samples in molecular Clade I for which chromosome counts were available were $n = 10$ in *D. elegans* and $n = 6$, 8 or 10 in *D. yina*. *Downingia yina* counts of $n = 6$ and $n = 8$ were documented from Marion and Lane counties in Oregon, respectively (Weiler 1962; Foster 1972). All samples in molecular Clade II were *D. yina* with $n = 10$. All samples in molecular Clade III were $n = 12$ in either *D. yina* or *D. bacigalupii*. Character state reconstruction suggests an ancestral state of $n = 10$ in Clades I and II, and an ancestral state of $n = 12$ in Clade III. The ancestral state for the entire *D. yina* complex is equivocal.

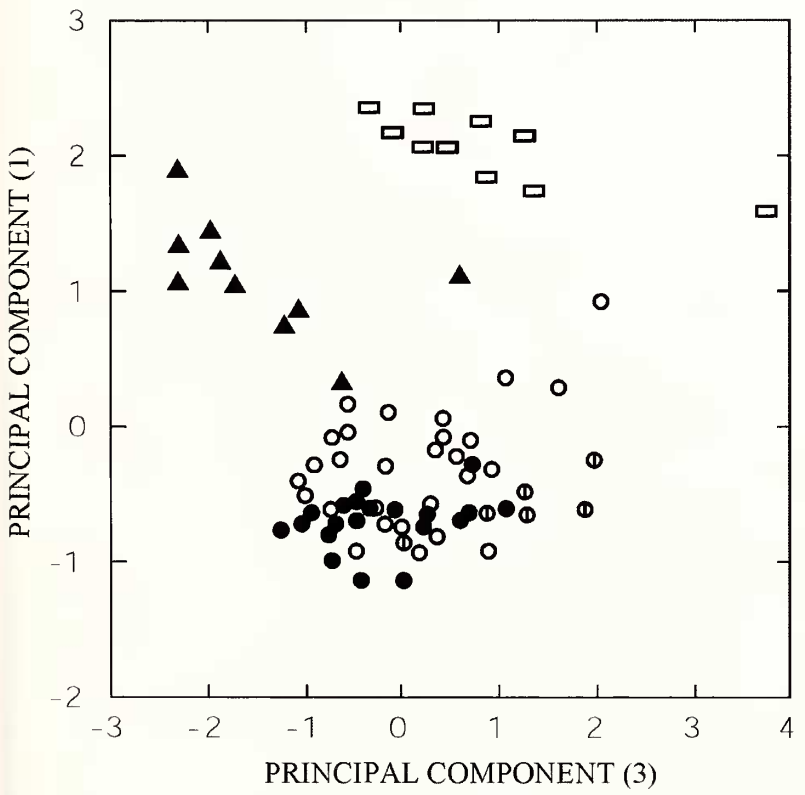

FIG. 6. Plot of principal components one and three using the characters listed in Table 1. Filled symbols represent Clade I. Symbols with a line through the center represent Clade II. Open symbols represent Clade III. Triangles = *Downingia elegans*. Squares = *D. bacigalupii*. Circles = *D. yina*.

Interfertility

Foster's results (1972) show that within *D. yina*, crosses between populations with different chromosome numbers showed a significant reduction in pollen stainability relative to crosses between populations with the same chromosome numbers (Table 5; Foster 1972). Similarly, pollen stainability was significantly reduced in crosses between populations presumed to be from different molecular clades relative to those presumed to be from the same molecular clades (Table 5; Foster 1972).

Weiler's results (1962) from interspecific reciprocal crosses (Table 6) reflect Foster's results within *D. yina* in that pollen stainability and meiotic irregularities seemed to be affected more by differences in chromosome number than by species identification (*D. elegans*, *D. bacigalupii*, or *D. yina*). Crosses between *D. elegans* ($n = 10$) and $n = 10$ populations of *D. yina*, for example, produced 10 bivalents and no significant reductions in pollen stainability (Table 6), in contrast to the reduction in pollen stainability for crosses within *D. yina* but between populations of different chromosome number (Table 5; Foster 1972).

TABLE 4. UNIVARIATE STATISTICS WITHIN *DOWNINGIA YINA*. Means, standard deviations, and ranges (in parentheses) are provided for each character within inferred molecular clades. Superscripts indicate groups that are significantly different from one another using Tukey multiple comparison tests following ANOVA. Groups with no superscript or that share a superscript are not significantly different.

Character	Clade I (n = 100)	Clade II (n = 30)	Clade III (n = 187)
Sepal (mm)	4.78 ± 1.03^{AB} (3.0–7.75)	4.22 ± 0.96^{B} (0.5–6.0)	4.82 ± 1.42^{A} (2.0–10.0)
Side slit (mm)	4.18 ± 0.63^{A} (2.75–6.0)	3.40 ± 0.49^{B} (2.75–4.5)	4.17 ± 0.73^{A} (1.75–6.0)
Back slit (mm)	4.86 ± 0.78^{A}(3.25–7.0)	3.74 ± 0.53^{B} (3.0–5.0)	4.94 ± 0.88^{A} (2.25–7.5)
Upper lobe (mm)	3.41 ± 0.71^{B} (2.0–5.75)	3.44 ± 0.81^{B} (2.0–5.0)	3.90 ± 0.99^{A} (2.0–6.75)
Lower lobe (mm)	6.00 ± 0.96^{C} (3.25–8.5)	5.09 ± 0.99^{B} (3.25–6.75)	6.46 ± 1.39^{A} (3.0–9.5)
Filament (mm)	2.99 ± 0.62^{C} (2.0–4.75)	2.33 ± 0.45^{B} (1.75–3.5)	3.40 ± 1.01^{A} (1.25–5.75)
Anther (mm)	2.12 ± 0.33^{C} (1.25–2.75)	1.75 ± 0.33^{B} (1.25–2.25)	2.28 ± 0.32^{A} (1.25–3.0)
Anther angle (degrees)	18.72 ± 8.10^{B} (2.0–40.0)	22.50 ± 6.11^{AB} (7.0–33.0)	23.96 ± 9.52^{A} (0.0–51.0)
Lower angle (degrees)	59.42 ± 12.24^{B} (35.0–90.0)	46.27 ± 12.40^{A} (20.0–68.0)	52.06 ± 12.08^{A} (22.0–78.0)
Horns (mm)	0.38 ± 0.08^{B} (0.19–0.61)	0.34 ± 0.04^{B} (0.26–0.42)	0.42 ± 0.13^{A} (0.13–0.77)
Filament/anther	1.42 ± 0.23 (1.0–2.0)	1.37 ± 0.30 (0.875–2.2)	1.48 ± 0.34 (0.625–2.375)
Side slit/back slit	0.86 ± 0.08^{A} (0.62–1.07)	0.91 ± 0.09^{B} (0.77–1.25)	0.85 ± 0.10^{A} (0.47–1.31)
Filament/back slit	0.61 ± 0.08^{B} (0.5–0.9)	0.62 ± 0.08^{AB} (0.47–0.75)	0.69 ± 0.22^{A} (0.38–2.0)
Upper lobe/lower lobe	0.58 ± 0.12^{C} (0.33–0.9)	0.68 ± 0.13^{B} (0.45–0.92)	0.62 ± 0.15^{A} (0.31–1.3)
Back slit/upper lobe	1.50 ± 0.44^{B} (0.78–3.11)	1.17 ± 0.41^{A} (0.60–2.22)	1.34 ± 0.38^{A} (0.50–2.5)

DISCUSSION

The *Downingia yina* species complex currently comprises three species that are readily distinguished from one another on the basis of morphological characteristics (Weiler 1962; Ayers 1993; Fig. 6, Table 3). *Downingia elegans* and *D. bacigalupii* differ from *D. yina* in that the anthers form a sharp angle relative to the filaments, and the lower corolla lobes are relatively parallel versus divergent in *D. yina*. The chromosome numbers and the yellow patches on the lower corolla lobes readily distinguish *D.bacigalupii* from *D. elegans*. As outlined in the introduction, previous workers (Peck 1934, 1937; McVaugh 1941, 1943) recognized that *D. yina* may represent multiple taxa, which were variously named: *D. yina* Applegate, *D. yina* Applegate var. *major* McVaugh, *D. willamettensis* Peck, *D. pulcherrima* Peck. Recent molecular analyses lent merit to these interpretations, but sampling within the *D. yina* complex was very limited (Schultheis 2001). The additional molecular data presented here substantiates these patterns, and demonstrates that samples of *D. yina* fall into three separate molecular clades, with *D. elegans* and *D. bacigalupii* nested within two of these three clades (Figs. 2–4). Taken independently, this paraphyletic or polyphyletic pattern with respect to the sequence data from either the nuclear or chloroplast genomes (Figs. 2–4) might only represent gene rather than organismal phylogenies (Doyle 1992; Knox 1998). High resolution molecular data are expected to reveal patterns in which paraphyletic progenitor species (with respect to the molecular data) give rise to monophyletic derivative species (Rieseberg and Brouillet 1994; Graybeal 1995; Olmstead 1995), in this case *D.*

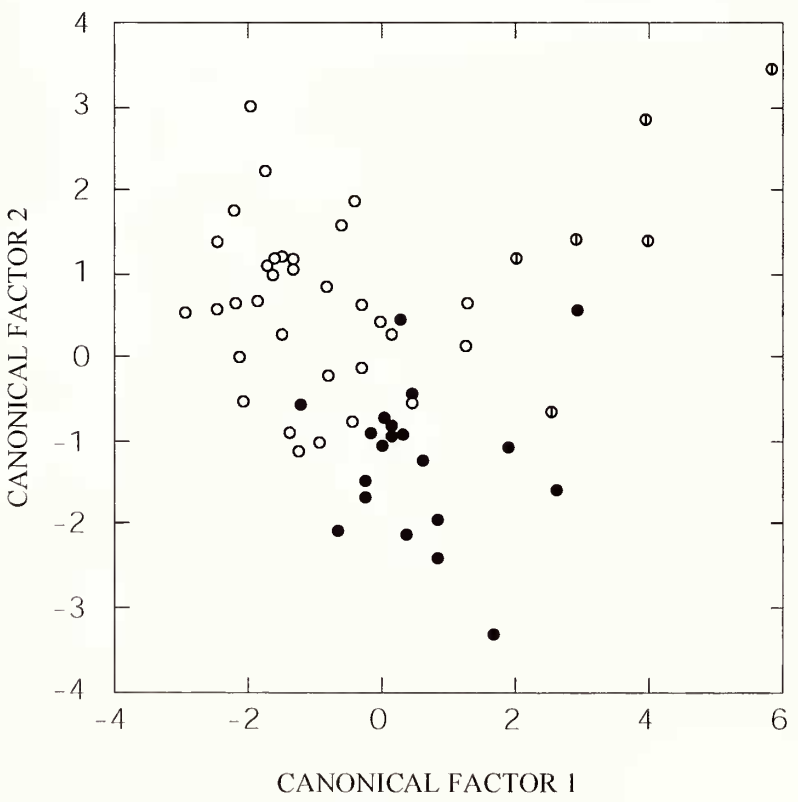

FIG. 7. Plot of canonical factors one and two from discriminant function analysis of *Downingia yina* samples using the characters listed in Table 1. Filled circles = Clade I. Circles with a line through the center = Clade II. Open circles = Clade III.

TABLE 5. MEAN PERCENT STAINABLE POLLEN IN CROSSES BETWEEN POPULATIONS OF *DOWNINGIA YINA*. Significant differences occur for populations with the same versus different chromosome numbers and for populations from the same versus different molecular clades. (Raw data taken from Foster (1972) and reanalyzed).

Cross type	Mean	SE	n	P value
Chromosome numbers same	93.4	8.4	5	0.001
Chromosome numbers differ	49.9	5.6	11	
Same molecular clade	83.0	8.3	7	0.007
Different molecular clades	48.3	7.3	9	

TABLE 6. MEAN PERCENT STAINABILITY AND MEIOTIC CONFIGURATIONS IN INTERSPECIFIC CROSSES WITHIN THE *DOWNINGIA YINA* COMPLEX. Data taken from Weiler (1962).

	D. elegans	*D. bacigalupii*
D. elegans	>95%	
$n = 10$		
D. bacigalupii	30.8–78.3%	>95%
$n = 12$	1ch3 + 9II + 1I	
D. yina	51.3–79.5%	>95%
$n = 12$	12II	
D. yina	>95%	50–70%
$n = 10$	10II	1ch3 + 9II + 1I

yina independently giving rise to *D. elegans* and *D. bacigalupii*. If *D. yina* populations were integrated through gene flow with one another, but to the exclusion of *D. elegans* and *D. bacigalupii*, *D. yina* would eventually proceed to monophyly with respect to the molecular data, and the currently recognized species would be appropriate, or could be accommodated with terms indicating their unresolved or transitional status ("metaspecies", Donoghue 1985; "ferre-species", Graybeal 1995; "plesiospecies", Olmstead 1995). What is compelling in this example is the correspondence of gene geneologies from more than one gene with geographic, cytological, and interbreeding data; a correspondence that makes a case for multiple organismal lineages (Avise 1994), and thus multiple species (de Queiroz 1998, 1999) within *D. yina*.

Geography

In the *D. yina* complex, cytological races and molecular clades appear to be roughly segregated along the Cascade Ranges (Fig. 1). When a concordant pattern emerges between phylogenetic and geographic subdivisions of a group, this often indicates little to no gene flow among subdivisions. This point has been emphasized in phylogeographic studies (Avise et al. 1987) and has received confirmation from population genetic models (Slatkin 1989). The correspondence between molecular clades within the *D. yina* complex and the distribution of these clades to either the west or east of the Cascade Ranges is striking (Figs. 1–5), and suggests that the mountain range serves as a geographic barrier to gene flow. The Cascade Ranges have been recognized as a geographic barrier in other contexts, clearly affecting differences in climate (Peck 1941; Orr and Orr 1996), and floristic composition (Peck 1941) to the west versus the east. The Klamath-Siskiyou region at the California-Oregon border is where the striking segregation of *D. yina* molecular clades to the east and west of the Cascade Ranges is much less evident (Fig. 1). Clade I is found to the west of the Cascade Ranges, except that *D. elegans* extends eastward into eastern Washington and Idaho. Clade II is localized to a region in the Cascade Range of southern Oregon (Fig. 1), in the vicinity of Lake of the Woods and Upper Klamath Lake, Oregon, and cannot readily be designated as "east" or "west". Molecular clade III is primarily east of the Cascades, but extends west into the Klamath-Siskiyou region. It is possible that the Klamath-Siskiyou region was the source from which the *D. yina* complex dispersed northward to the east and west of the Cascades, a scenario similar to hypotheses of post-glaciation dispersal presented by Whittaker (1961) and Soltis et al. (1997).

Cytology

Cytological variation within the *D. yina* complex mirrors the molecular phylogeny and the geography for the group, with $n = 12$ samples primarily east of the Cascade Ranges, and $n = 10$ samples primarily to the west. Populations of *D. yina* within Clade I have $n = 6$ or 8 in the northwestern reaches of the species range, an observation which prompted Foster (1972) to suggest a trend of decreasing chromosome numbers as one progressed from the southeast to the northwest of *D. yina*'s range. Foster's (1972) proposed explanation for this trend, based on meiotic configurations in numerous hybrids between the different chromosome races of *D. yina*, was that the races arose through Robertsonian translocations producing either a dysploid series of reductions from a starting point of $n = 12$, or a series of reductions from $n = 11$ with an increase to $n = 12$. Foster's work (1972) unfortunately did not include *D. elegans* and *D. bacigalupii*, perhaps because the potential derivation of these taxa from within *D. yina* was not reflected in the taxonomy. If *D. elegans* and *D. bacigalupii* arose from within *D. yina*, as suggested by the molecular data, the simplest explanation is that they arose from $n = 10$ and $n = 12$ populations of *D. yina*, respectively. The homology of *D. elegans* and $n = 10$ *D. yina* genomes, and of *D. bacigalupii* and $n = 12$ genomes is supported by interfertility data discussed below.

Interfertility

If *D. yina* contains the multiple divergent lineages suggested by the molecular data, one might expect levels of interfertility to correspond with the molecular clades. Indeed, levels of interfertility appear to correspond more with the molecular clades and the chromosome numbers of the populations examined than with species identification. For example, individuals of *D. yina* from Clade I show greater interfertility with *D. elegans* than with individuals of *D. yina*

from Clade III (Tables 6 and 7). Similarly, individuals of *D. yina* from Clade III show greater interfertility with *D. bacigalupii* than with individuals of *D. yina* from Clades I or II. In sum, patterns of interfertility do not appear to correspond to the species currently recognized, but do appear to correspond to chromosome races and molecular data, both of which correspond to geography.

While levels of fertility may be reduced in crosses between chromosome races or molecular clades, reproductive barriers are not complete. Nor are reproductive barriers complete among the three species currently recognized. Populations exist with hybrids between *D. bacigalupii* and *D. yina*, and between *D. elegans* and *D. yina* (Weiler 1962; Schultheis personal observation). These populations may either resemble a hybrid swarm, with a wide variety of hybrid forms, or may contain readily distinguishable parental forms and only a few hybrids (Weiler 1962; Schultheis personal observation). Regardless of whether reproductive barriers are complete or incomplete, the currently recognized species of the *D. yina* complex do not correspond to patterns of interfertility within the group.

Hypothesized Organismal Lineages Within the *Downingia yina* Complex

In sum, there appear to be three main lineages within the *D. yina* species complex. Members of the first lineage (Clade I) are characterized by either a "*D. yina*" or "*D. elegans*" morphology, and are distributed primarily west of the Cascades, with *D. elegans* extending eastward into eastern Washington and Idaho. "*D. yina*" individuals are $n = 6$, 8, or 10. "*D. elegans*" individuals are $n = 10$. Within this lineage, the "*D. elegans*" members form a clade, excluding sample *Foster 70-15-4*, from the southern periphery of the "*D.elegans*" range. The scant support for the "*D. elegans*" clade comes from morphological characters, some of which are polymorphic within populations (Table 2).

The second hypothesized lineage (Clade II), localized in the Lake of the Woods region of the Cascades in southern Oregon, is characterized by a "*D. yina*" morphology and $n = 10$. Support for this clade comes entirely from molecular characters.

Members of the third hypothesized lineage (Clade III) are characterized by either a "*D. yina*" or "*D. bacigalupii*" morphology, $n = 12$, and a distribution primarily to the east of the Cascades, into southwestern Idaho and western Nevada, and extending westward into the Klamath/Siskiyou region of southern Oregon and northern California. Within this lineage, the "*D. bacigalupii*" samples form a clade to the exclusion of sample 585-99, from the western periphery of the range. The "*D. bacigalupii*" clade is supported only by morphological characters (Table 2).

Morphological analyses presented here were unable to clearly distinguish among *D. yina* samples falling into different molecular clades (Fig. 7; Table 4), which largely correspond to variation in *D. yina* chromosome numbers. Similarly, Foster (1972) was unable to find morphological differences corresponding to the chromosome races within *D. yina*. The chromosome races and the molecular clades within *D. yina* are morphologically cryptic. Further examination of morphology may reveal differences missed thus far, but even in the absence of such differences, it is desirable to recognize what are hypothesized to be organismal lineages.

Based on the information currently available, I choose to recognize five species, with names assigned based on nomenclatural priority and the phylogenetic placement of the type specimens: *D. elegans* (Lindley) Torrey, *D. bacigalupii* Weiler, *D. yina* Applegate, *D. willamettensis* Peck, and *D. pulcherrima* Peck. Ideally taxon names, including species names, should only be assigned to clades (Mishler and Donoghue 1982; Misher and Theriot 2000). This strict application of a phylogenetic species concept only applies full species status to *D. elegans*, *D. bacigalupii*, and *D. yina sensu stricto*. *Downingia willamettensis* and *D. pulcherrima* comprise the "*D. yina*" samples from Clades I and III respectively. These samples are not resolved as clades, but may still be named as metaspecies (Donoghue 1985), plesiospecies (Olmstead 1995) or ferrespecies (Graybeal 1995). An alternative to recognizing five species is to recognize a single species, *D. elegans* (based on nomenclatural priority), and five varieties. While both alternatives recognize the same taxa, differing only in the rank applied (species or variety), the recognition of five species more clearly emphasizes the molecular, cytological and fertility diversity within this complex group. In this case, names are also available at the species rank whereas new names or combinations would be needed if the taxa were recognized at the varietal rank.

Features of the five taxa are summarized in Table 7. It is unfortunate that the three species previously referred to *D. yina* (*D. yina* s.s., *D. willamettensis*, and *D. pulcherrima*) are morphologically indistinguishable given current information. Even those features of most importance in discriminate function analysis (included in Table 7) show such overlap as to be of minimal use for field identification. Weiler (1962) did note that *D. yina* tended to be decumbent in the west and erect in the east (which would correspond to *D. willamettensis* and *D. pulcherrima* respectively), but this can be difficult to detect on herbarium sheets. This feature, as well as corolla coloration (particularly useful for distinguishing *D. elegans* and *D. bacigalupii*), is worth noting in

TABLE 7. SUMMARY OF THE FEATURES DISTINGUISHING THE FIVE SPECIES FORMERLY CLASSIFIED AS *DOWNINGIA ELEGANS*, *D. BACIGALUPII* AND *D. YINA*. * Indicates features identified in discriminant function analysis within *D. yina*. Mean ± standard deviation; based on measurements from samples in this study (Appendix 1).

	D. elegans	*D. bacigalupii*	*D. yina*	*D. willamettensis*	*D. pulcherrima*
Former classification	*D. elegans*	*D. bacigalupii*	*D. yina*	*D. yina*	*D. yina*
Cytology	$n = 10$	$n = 12$	$n = 10$	$n = 6, 8, 10$	$n = 12$
Elevation	<2000 m	<2000 m	1200–1510 m	<250 m; 650 m in Lake Co., CA	generally <2000 m
Geographic distribution	W of Cascades in Oregon and Washington; W of North Coast Ranges in California; extending eastward into eastern Washington and Idaho	E of Cascades in California and Oregon; extending westward into Klamath-Siskiyou region of northern California and southern Oregon; southwestern Idaho and western Nevada	localized to Cascades of southern Oregon, between northwestern Upper Klamath Lake and Lake of the Woods	W of Cascades in Washington and Oregon; W of North Coast Ranges in northwestern California	E of Cascades in Washington, Oregon, and California; extending westward into Klamath-Siskiyou region of northern California and southern Oregon
Morphology					
Anther angle relative to filament tube	sharply bent (84.1 degrees ± 14.4)	sharply bent (88.8 degrees ± 6.7)	not sharply bent (22.5 degrees ± 6.1)	not sharply bent (18.7 degrees ± 8.1)	not sharply bent (24.0 degrees ± 9.5)
Lower corolla lobes	nearly parallel (9.2 degrees ± 11.9)	nearly parallel (12.3 degrees ± 9.9)	divergent (46 degrees ± 12)*	divergent (59 degrees ± 12)*	divergent (52 degrees ± 12)*
Yellow in corolla throat.	absent	present	present	present	present
Filament tube	5.3 mm ± 1.5	7.5 mm ± 1.5 (longer on average than other species)	2.3 mm ± 0.5	3.0 mm ± 0.6	3.4 mm ± 1.0
Anther length	2.7 mm ± 0.5	2.9 mm ± 0.4	1.75 mm ± 0.3*	2.1 mm ± 0.3*	2.3 mm ± 0.3*
Trichomes on anther dorsal surface	generally few (can be none to abundant)	generally few (can be none to abundant)	generally less abundant than *D. willamettensis* and *D. pulcherrima* (can be none to abundant)*	generally more abundant than *D. yina* and *D. pulcherrima* (can be none to abundant)*	generally few (can be none to abundant)*

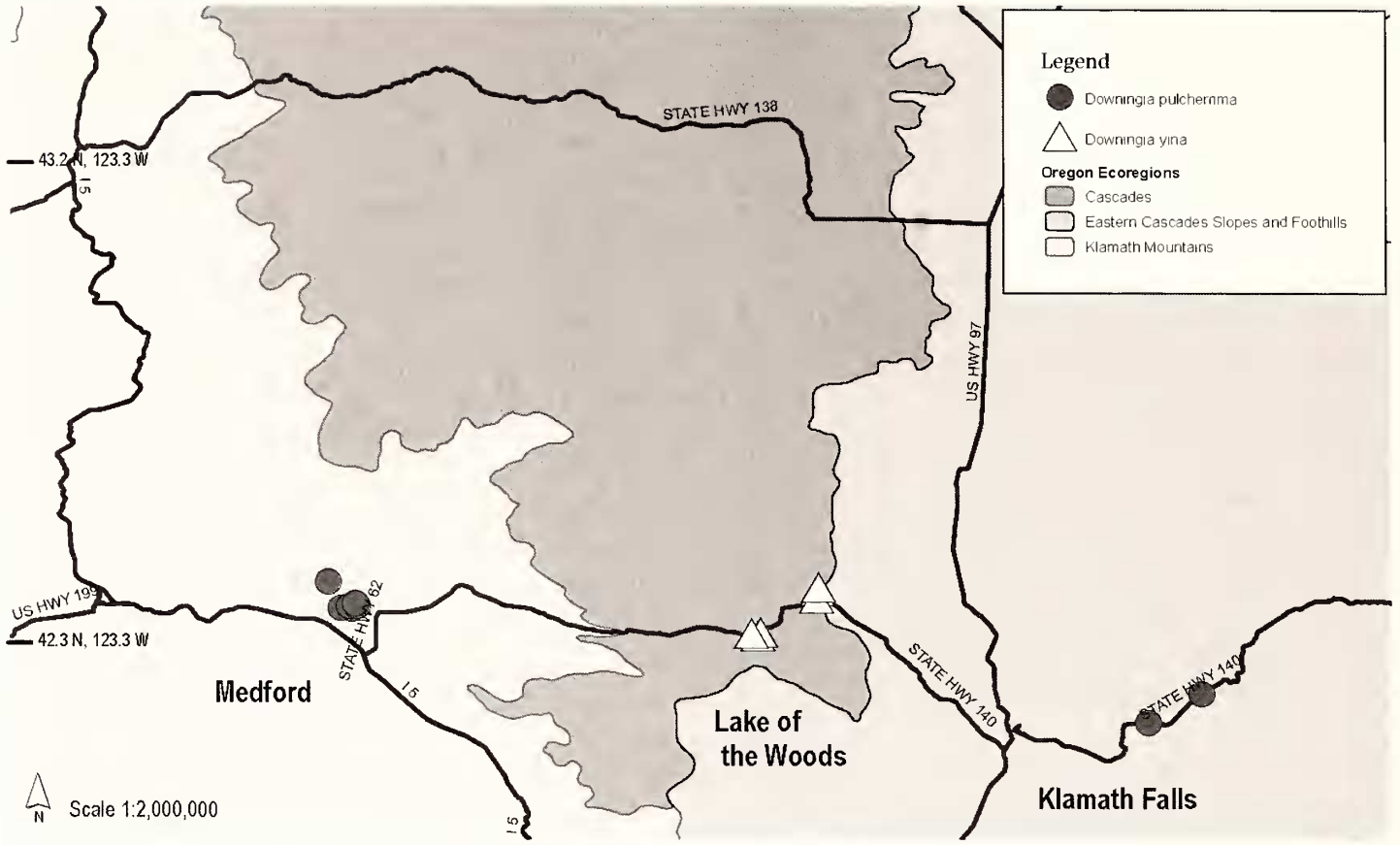

FIG. 8. Map illustrating the distribution of *Downingia yina sensu strictu* relative to adjacent *D. pulcherrima* populations in the southern Cascade Range of Oregon. The map does not illustrate *D. bacigalupii*, which is also found in the pictured region. Triangles = *D. yina* s.s. (n = 10, Clade II). Circles = *D. pulcherrima* (n = 12, Clade III). *Downingia yina* s.s. samples are located within the southern tip of the Cascades ecoregion of Oregon (following Thorson et al. 2003).

future collections. Unless reliable features are identified, we must rely on geographic location for field identification, ideally with confirmation from molecular and/or cytological data. At present I recommend that specimens collected west of the Cascades in Oregon and Washington, and west of the North Coast Ranges in California are best assigned to *D. willamettensis*. Specimens collected east of the Cascades in Oregon or Washington are best assigned to *D. pulcherrima*. *Downingia pulcherrima* is also located in the Klamath and Siskiyou regions of northern California (documented in this study as far west as Coffee Creek, just west of Clair Eagle Lake, Trinity Co.) and southern Oregon (documented in this study as far west as Medford, Jackson Co.). *Downingia pulcherrima* and *D. willamettensis* are generally above and below elevations of 250 m respectively. *Downingia yina sensu strictu* is localized to the southern tip of the Cascade Range in Oregon. This study documents populations from the northwestern edge of Upper Klamath Lake to Lake of the Woods (Klamath Co.). Based on my current understanding of the distribution for *D. yina*, I recommend assigning to this taxon any collections found in the Cascades ecoregion of southern Oregon (ecoregion as delimited in Thorson et al. 2003), while assigning those found in neighboring areas outside of this ecoregion to *D. pulcherrima*. Figure 8 provides a map delimiting the distribution of *D. yina* relative to *D. pulcherrima*.

Priorities for refining our current understanding of this species complex include obtaining molecular data from additional populations (particularly at the limits of species ranges, including Washington state) additional sampling of cytological variation, and exploration of morphological or ecological features to distinguish *D. yina sensu strictu*, *D. willamettensis*, and *D. pulcherrima*.

Key to Taxa of the *Downingia yina* Species Complex

1a. Anthers abruptly bent, >70° to filaments; lower corolla lip lobes ± parallel.
  2a. Corolla 3-colored (blue, white, yellow); lower corolla lobes obtuse, mucronate . . . . . *D. bacigalupii*
  2b. Corolla 2-colored (blue, white); lower corolla lobes acute . . . . . *D. elegans*
1b. Anthers not or ± bent, <45° to filaments; lower corolla lip lobes divergent, not parallel.
  3a. Plants generally east of Cascades, extending into Klamath Ranges in southern Oregon and northern California; generally >250 m (but <250 m along Columbia River, Washington).
    4a. Localized to southern Oregon Cascades, between northwestern Upper Klamath Lake and Lake of the Woods, plants at 1200–1510 m . . . *D. yina*
    4b. East of Cascades, extending into Klamath Ranges in southern Oregon and northern California, plants generally at <2000 m . . . . . *D. pulcherrima*

3b. Plants generally west of Cascades in Oregon and Washington, and west of North Coast Ranges in California; generally <250 m (but 650 m on Snow Mountain, Lake Co., California) . . . . . *D. willamettensis*

Acknowledgments

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Literature Cited

Applegate, E. I. 1929. Two new *Downingias* from Oregon. Contributions from the Dudley Herbarium of Stanford University 1:97–98.

Archie, J. W. 1985. Methods for coding variable morphological features for numerical taxonomic analysis. Systematic Zoology 34:326–345.

Avise, J. C. 1994. Molecular markers, natural history and evolution. Chapman & Hall, New York, NY.

———, J. Arnold, R. M. Ball, Jr., E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific phylogeography: the mitochrondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics 18:89–522.

Ayers, T. 1993. *Downingia*. Pp. 460–462 *in* J. C. Hickman (ed.), The Jepson manual: higher plants of California. University of California Press, Berkeley, CA.

Baldwin, B. G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. Molecular Phylogenetics and Evolution 1:3–16.

Bauder, E. 1992. Ecological monitoring of *Downingia concolor* ssp. *brevior* (Cuyamaca Lake downingia) and *Limnanthes gracilis* ssp. *parishii* (Parish's slender meadowfoam). Report numbers 844-06-328, FG 7414, FG 9443. California Department of Parks and Recreation Southern Division, and California Department of Fish and Game Natural Heritage Division, Sacramento, CA.

Bremer, K. 1994. Branch support and tree stability. Cladistics 10:295–304.

De Queiroz, K. 1998. The general lineage concept of species, species criteria, and the process of speciation: a conceptual unification and terminological recommendation. Pp. 57–78 *in* D. J. Howard and S. H. Berlocher (eds.), Endless forms: species and speciation. Oxford University Press, New York, NY.

———. 1999. The general lineage concept of species and the defining properties of the species category. Pp. 49–89 *in* R. A. Wilson (ed.), Species: new interdisciplinary essays. The MIT Press, Cambridge, MA.

Donoghue, M. J. 1985. A critique of the biological species concept and recommendations for a phylogenetic alternative. Bryologist 88:172–181.

———, R. G. Olmstead, J. F. Smith, and J. D. Palmer. 1992. Phylogenetic relationships of Dipsacales based on *rbc*L sequences. Annals of the Missouri Botanical Garden 79:249–265.

Doyle, J. J. 1992. Gene trees and species trees: molecular systematics as one-character taxonomy. Systematic Botany 17:144–163.

——— and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19:11–15.

Eriksson, T. 1998. AutoDecay, ver. 4.0 (program distributed by author). Department of Botany, Stockholm University, Stockholm, Sweden.

Farris, J. S., M. Källersjö, A. G. Kluge, and C. Bult. 1995. Testing significance of incongruence. Cladistics 10:315–319.

Foster, R. I. 1972. Explosive chromosome evolution in *Downingia yina*. Ph.D. dissertation. University of California, Davis, CA.

Graybeal, A. 1995. Naming species. Systematic Biology 44:237–250.

Hillis, D. M., B. K. Mable, A. Larson, S. K. Davis, and E. A. Zimmer. 1996. Nucleic acids IV: sequencing and cloning. Pp. 321–381 *in* D. M. Hillis, C. Moritz, and B. K. Mable (eds.), Molecular systematics, 2nd ed. Sinauer Associates, Inc., Sunderland, MA.

Knox, E. 1998. The use of hierarchies as organizational models in systematics. Biological Journal of the Linnean Society 63:1–49.

Küchler, A. W. 1964. Potential natural vegetation of the conterminous United States. American Geographical Society Special Publication no. 36. American Geographical Society, New York, NY.

Lammers, T. G. 1993. Chromosome numbers of Campanulaceae. III. Review and integration of data for subfamily Lobelioideae. American Journal of Botany 80:660–675.

Maddison, W. P. and D. R. Maddison. 1992. MacClade: analysis of phylogeny and character evolution, ver. 3.0. Sinauer, Sunderland, MA.

McVaugh, R. 1941. A monograph of the genus *Downingia*. Memoirs of the Torrey Botanical Club 19:1–57.

———. 1943. Campanulaceae (Lobelioideae). North American Flora 32A:1–134.

Mishler, B. D. and M. J. Donoghue. 1982. Species concepts: a case for pluralism. Systematic Zoology 31:491–503.

——— and E. Theriot. 2000. The phylogenetic species concept *sensu* Mishler and Theriot: monophyly, apomorphy, and phylogenetic species concepts. Pp. 44–54 *in* Q. D. Wheeler and R. Meier (eds.), Species concepts and phylogenetic theory: a debate. Columbia University Press, New York, NY.

Olmstead, R. G. 1995. Species concepts and plesiomorphic species. Systematic Botany 20:623–630.

Orr, E. L. and W. N. Orr. 1996. Geology of the Pacific Northwest. The McGraw-Hill Companies, Inc., New York, NY.

Peck, M. E. 1934. New Oregon plants. Proceedings of the Biological Society of Washington 47:185–188.

———. 1937. New plants from Oregon. Proceedings of the Biological Society of Washington 50:93–94.

———. 1941. A manual of the higher plants of Oregon. Binford & Mort, Publishers, Portland, OR.

RIESEBERG, L. H. AND L. BROUILLET. 1994. Are many plant species paraphyletic? Taxon 43:21–32.

SCHULTHEIS, L. M. 2001. Systematics of *Downingia* (Campanulaceae) based on molecular sequence data: implications for floral and chromosome evolution. Systematic Botany 26:603–621.

SLATKIN, M. 1989. Detecting small amounts of gene flow from phylogenies of alleles. Genetics 121:609–612.

SOLTIS, D. E., M. A. GITZENDANNER, D. D. STRENGE, AND P. S. SOLTIS. 1997. Chloroplast DNA intraspecific phylogeography of plants from the Pacific Northwest of North America. Plant Systematics and Evolution 206:353–373.

SWOFFORD, D. L. 1993. PAUP: phylogenetic analysis using parsimony, version 3.1. Computer program distributed by the Illinois Natural History Survey, Champaign, IL.

———. 2001. PAUP*: phylogenetic analysis using parsimony. Version 4. Sunderland: Sinauer Associates, Inc. Publishers, Sunderland, MA.

THORSON, T. D., S. A. BRYCE, D. A. LAMMERS, A. J. WOODS, J. M. OMERNIK, J. KAGAN, D. E. PATER, AND J. A. COMSTOCK. 2003. Ecoregions of Oregon (color poster with map, descriptive text, summary tables, and photographs): map scale 1:1,500,000, U.S. Geological Survey, Reston, VA.

WEILER, J. H., JR. 1962. A biosystematic study of the genus *Downingia*. Ph.D. dissertation. University of California, Berkeley, CA.

WHITTAKER, R. H. 1961. Vegetation history of the Pacific Coast states and the "central" significance of the Klamath Region. Madroño 16:5–23.

WOOD, C. W., JR. 1961. A study of hybridization in *Downingia* (Campanulaceae). Journal of the Arnold Arboretum 2:219–262.

APPENDIX 1

SPECIMEN VOUCHERS USED IN CURRENT STUDY, GROUPED UNDER SPECIES AND STATE. Specimens used in the morphological analysis are indicated by an asterisk. GenBank sequence accession number(s) are given for specimens used in the molecular analysis and haploid chromosome number for specimens used to collect chromosome data. Unmounted collections by Foster are deposited at DAV, accessioned collections are at JEPS or UC.

Voucher	GenBank sequence	Chromosome number
Downingia bacigalupii		
CALIFORNIA		
* *Schultheis 233-95*, Lassen Co: SW of Nubeiber, Hwy 299 at entrance to Muck Valley Hydroelectric Project (JEPS)		
* *S. Jessup s.n.*, Modoc Co.: N of Canby, 1.4 mi N of Rt 139 on Rt 46 (JEPS)		
Schultheis 230-95, Modoc Co.: N of Lookout, on Cty Rd 94, 0.3 mi S of Cedar Dr. (JEPS)	AF163376, AF163377	
* *Schultheis 231-95*, Modoc Co.: N of Lookout, at Cty Rd 94 and railroad crossing (JEPS)		
* *Schultheis 234-95*, Shasta Co.: Rt 89, 3.2 mi S of Shasta-Siskiyou county line (JEPS)		
* *Schultheis 252-95*, Sierra Co.: Sierra Valley, SW corner of road to Calpine and Rd A-23 (JEPS)		
* *Schultheis 251-95*, Sierra Co.: Sierra Valley, road A-23, 0.7 mi N of turn off to Calpine (JEPS)	AF176900, AF176878	
OREGON		
* *Schultheis 240-95*, Harney Co.: On Hwy 20, 1 mi N of junction with 395, N of Riley (JEPS)	AF163382, AF163383, AF176888	
* *Schultheis 582-99*, Jackson Co.: N of Medford, NE corner of Table Rock and Kirtland Rds. (JEPS);		
* *Schultheis 585-99*, Josephine Co.: Botanical Wayside Park at Rough and Ready Creek, S of Caves Junction (JEPS)	AF229132, AF229133, AF229151	
* *Weiler 61323*, Josephine Co.: 0.4 mi W of old site of Waldo (UC)		
* *Weiler 61319*, Josephine Co.: Hwy 199, 3.7 mi N of O'Brien (UC)		
* *Schultheis 237-95*, Klamath Co.: Rt 66, 5 mi W of Keno (JEPS)	AF229136, AF229137, AF229149	
* *Weiler 61452*, Klamath Co.: Pelican Ranger Station, 3 mi SW of Rocky Point (UC)		
Downingia elegans		
CALIFORNIA		
* *Tracy 15462*, Humboldt Co.: Larrabee Valley (UC)		
* *Weiler 60138*, Lake Co.: Snow Mountain, 3.5 mi N of Bear Creek Public Camp (UC)		$n = 10$ (Cited in Weiler 1962)
Foster 70-15-4, Lake Co.: Snow Mountain, near Kelly Cabin Site (DAV)	AF229140, AF229141, AF229152	$n = 10$ (Cited in Foster 1972 as "Snow Mt.")
* *Weiler 60256*, Mendocino Co.: S limits of Willits, E of Hwy 101 (UC)		
* *Tracy 17402*, Trinity Co.: Hettenshaw Valley (UC)		
IDAHO		
* *Aller 3000*, Benewah Co.: 1.5 mi S of Tensed (UC)		
* *Ehlers & Erlanson 39*, Bonner Co.: Edge of Lake Pend Oreille (UC)		
* *Constance 2025*, Clearwater Co.: 5 mi W of Weippe (UC)		

APPENDIX 1. CONTINUED.

Voucher	GenBank sequence	Chromosome number
OREGON		
* *Schultheis 244-95*, Benton Co: William Finley Natl. Wildlife Reserve (JEPS)		
* *Schultheis 243-95*, Benton Co.: William Finley Natl. Wildlife Reserve (JEPS)	AF163386, AF163387, AF176889	
* *Schultheis 317-96*, Linn Co.: E of Corvallis, NW corner of Hwy 34 and Looney Lane (JEPS)		
* *Schultheis 242-95*, Linn Co.: Hwy 34, 6.5 mi E of Corvallis, 1 mi E of Oakville Rd, just E of Lake Creek (JEPS)	AF163385, AF176877	
* *Schultheis 318-96*, Linn Co.: Outside of Eugene, Rt 126, 1.7 mi E of Greenhill Rd. (JEPS)		
* *Schultheis 320-96*, Washington Co.: Edge of Banks, Hwy 47, 1.6 mi N of Hwy 6, just S of Creps Rd. (JEPS)	AF163401, AF176885	
Downingia yina		
CALIFORNIA		
* *Oswald & Ahart 3943*, Butte Co.: Soda Ridge Rd, 1.4 mi W of junction W Coon Hollow Rd. (UC)	AF229146, AF229147	
* *Schultheis 247-95*, Del Norte Co.: Hiouchi. Off of Hwy 199, corner of Hiouchi and Jedediah Rds. (JEPS)	AF163388, AF163389, AF176892	
* *Schultheis 587-99*, Del Norte Co.: Hiouchi. Off of Hwy 199, corner of Hiouchi and Jedediah Rds. (JEPS)		
* *Tracy 3781*, Humboldt Co.: Alton (UC)		
* *Tracy 3217*, Humboldt Co.: Near Hydesville (UC)		
Foster 70-96-11, Humboldt Co.: Horse pasture by Hydesville School (DAV)	AF163425	$n = 10$ (Cited in Foster 1972 as "Hydesville")
* *Tracy 19527*, Humboldt Co.: Bed of former Goose Lake, in Hydesville (UC)		
* *T. O'Brien s.n.*, Siskiyou Co.: Tennant. Tennant Rd, 6.0 mi E of Hwy 97 (JEPS)	AF163439, AF176887	
* *Schultheis 580-99*, Siskiyou Co.: Trailer Lane and I-5, edge of Weed (JEPS)		
* *Schultheis 236-95*, Siskiyou Co.: Trailer Lane and I-5, edge of Weed (JEPS)	AF229134, AF229135, AF229148	
* *Weiler 60207*, Siskiyou Co.: Hwy 99, 2.3 mi NW of Weed (UC)		$n = 12$ (Cited in Weiler 1962)
* *Bacigalupi 5937*, Siskiyou Co.: 2 mi N of Weed along Hwy 99 (UC)		
Foster s.n., Siskiyou Co.: 2 mi N of Weed along Hwy 99 (DAV)		$n = 12$ (Cited in Foster 1972 as "Weed")
* *Bacigalupi 5696*, Siskiyou, CA. Hwy 99, 1.5 mi WNW of Weed (UC)		
* *Parker 100*, Siskiyou Co.: Shovel Creek, above Beswick (UC)		
Foster s.n., Siskiyou Co.: Big Flat, along Coffee Creek (DAV)		$n = 12$ (Cited in Foster 1972 as "Big Flat")
* *Douglas Barbe 348*, Trinity Co.: Trinity-Siskiyou county line, 0.5 mi N of Big Flat (JEPS)	AF229112, AF229113, AF229114	
* *Wagnon 1657*, Trinity Co.: Big Flat, along Coffee Creek (UC)		
* *Tebbe 140*, Yolo Co.; Sacramento Valley, near Woodland (UC)		

APPENDIX 1. CONTINUED.

Voucher	GenBank sequence	Chromosome number
OREGON		
* *Holmgren & Holmgren 9645*, Deschutes Co.: Hwy 20, just N of Lake Co. line (UC)		
* *Applegate 6155*, Douglas Co.: 2 mi N of Dillard, 5 mi S of Roseburg (UC)		
* *Kimber 59*, Douglas Co.: Near Drain (UC)		
* *Weiler 61332*, Douglas Co.: Hwy 99, 3 mi S of Yoncalla (UC)		
* *Weiler 61333*, Douglas Co.: 0.5 mi W of Hwy 99, 3 mi S of Yoncalla (UC)		$n = 10$ (Cited in Weiler 1962)
* *Bacigalupi 7861*, Douglas Co.: 2.5 mi S of Yoncalla, along Hwy 99 at Yoncalla-Drain exit (JEPS)		
* *Bacigalupi 7862*, Douglas Co.: 1.5 mi S of Yoncalla, just E of Hwy 236, in Pleasant Valley (JEPS)		
Foster 68-51, Douglas Co.: Off southern Yoncalla-Drain exit from I-5 (DAV)	AF163421, AF163422, AF176886	$n = 10$ (Cited in Foster 1972 as "Yoncalla")
Foster s.n., Douglas Co.: Sutherlin-Nonpariel Rd, 0.5 mi E of Platt K Road (DAV)		$n = 10$ (Cited in Foster 1972 as "Sutherlin")
* *Schultheis 241-95*, Harney Co.: Rt. 20, 2.3 mi from Lake Co. line (JEPS)	AF163384, AF176881	
* *Peck 18919*, Harney Co.: Silver Creek Valley, 10 mi W of Riley		
Foster s.n., Harney Co.: 12.5 mi S of Riley on Hwy 395 (DAV)		$n = 12$ (Cited in Foster 1972 as "Wagontire")
* *Halse 4662*, Harney Co.: Hwy 20 between Squaw and Glass Buttes, 6.2 mi E of Lake Co. line		
* *Peck 20902*, Harney Co.: 7 mi N of Wagontire (UC)		
* *Heller 15750*, Harney Co.: Hwy 395, N of Wagontire (UC)		
Robbins 4047, Jackson Co.: N of Medford (UC)		$n = 12$ (Cited in Weiler 1962)
* *Peck 24821*, Jackson Co.: Camp White (UC)		
* *Schultheis 246-95*, Jackson Co.: N of Medford, Kirtland Rd, 1.9 mi W of Table Rock Rd. (JEPS)		
* *Weiler 61195*, Jackson Co.: N of Medford, Kirtland Rd, 0.8 mi W of CampWhite-TouVelle State Park (UC)		
* *Schultheis 582-99*, Jackson Co.: N of Medford, NE corner of Table Rock and Kirtland Rds. (JEPS)		
* *Schultheis 245-95*, Jackson Co.: N of Medford, NE corner of Table Rock and Kirtland Rds. (JEPS)		
* *Weiler 61197*, Jackson Co.: Near TouVelle State Park, 0.25 mi S of Bybee Bridge (UC)		
* *Schultheis 584-99*, Jackson Co.: N of Medford, Tresham Lane just W of Table Rock Rd. (JEPS)		
Foster 70-43-15, Jackson Co.: Medford, Tresham Lane, 1 mi N of Table Rock Rd. (DAV)	AF163423	$n = 12$ (Cited in Foster 1972 as "Medford")
Foster 71-1-14, Jackson Co.: N of Medford on Hwy 66 (DAV)	AF163426, AF163427	$n = 12$ (Cited in Foster 1972 as "Agate"
* *Bacigalupi 5702*, Klamath Co.: 5 mi NE of Dairy (JEPS)		
* *T. O'brien s.n.*, Klamath Co.: Great Meadows Recreation Site, W of Klamath Falls on Hwy 140 (JEPS)		
* *Maguire 26588*, Klamath Co.: 16 mi E of Klamath Falls along Hwy 66 (UC)		
Foster s.n., Klamath Co.: 3–4 mi W of Dairy on Hwy 140 (DAV)		$n = 12$ (Cited in Foster 1972 as "Dairy")
* *Weiler 61449*, Klamath Co.: N side of Lake of the Woods (UC)		

APPENDIX 1. CONTINUED.

Voucher	GenBank sequence	Chromosome number
Weiler 61200, Klamath Co.: Lake of the Woods (UC)		$n = 10$ (Cited in Weiler 1962)
Foster 70-84, Klamath Co.: Near Lake of the Woods campground (DAV)	AF163424	$n = 10$ (Cited in Foster 1972 as "Aspen"
* *Schultheis 581-99*, Klamath Co.: W of Upper Klamath Lake, West Side Rd, 0.4 mi past O'Neil Rd N of Hwy 140 (JEPS)	AF229138, AF229139, AF229150	
* *R. Bacigalupi 7978*, Klamath Co.: Plane landing strip at NE end of Lake of the Woods (JEPS)	AF229144, AF229145, AF229153	
* *Bacigalupi 7981*, Klamath Co.: Pelican Guard Station, just W of Pelican Bay, Upper Klamath Lake (JEPS)		
Weiler 61451, Klamath Co.: Pelican Guard Station (UC)		$n = 10$ (Cited in Weiler 1962)
* *McVaugh 6303*, Klamath Co.: 3 mi SW of Rocky Point, on Lake of the Woods Rd. (UC)		
* *Cook 962*, Lane Co.: W of Eugene, 11th St where crosses flats E of Fern Ridge Lake (UC)		$n = 8$ (Cited in Weiler 1962)
Foster 71-13, Lane Co.: Road to S. J. Quam Rock Quarry off Hwy 126, W of Eugene (DAV)	AF163428	$n = 8$ (Cited in Foster 1972 as "Quarry"
Weiler 61349, Marion Co.: 0.5 mi NW of Aumsville (UC)		$n = 6$ (Cited in Weiler 1962)
* *Bacigalupi 7874*, Marion Co.: 0.5 mi NW of Aumsville (JEPS)		
* *Weiler 61354*, Marion Co.: Salem, junction of Hwy 99 and Hwy 22 (UC)		$n = 6$ (Cited in Weiler 1962)
* *Bacigalupi 7879*, Marion Co.: E of Salem, junction of Hwy 99 and OR Rd 20 (JEPS)		
* *Peck 16291*, Marion Co.: Near Aumsville (UC)		
Foster 68-210, Marion Co.: Drained marsh by Aumsville Elementary School (DAV)	AF163420	$n = 6$ (Cited in Foster 1972 as "Aumsville")
* *Schultheis 319-96*, Sherman Co.: Hwy 97, 5 mi S of Grass Valley (JEPS)	AF163400, AF176884	
* *Hitchcock 25649*, Sherman Co.: 4 mi S of Grass Valley (UC)		
* *Weiler 61383*, Wasco Co.: Western limits of The Dalles (UC)		$n = 12$ (Cited in Weiler 1962)
* *R. Bacigalupi 7894*, Wasco Co.: Western edge of The Dalles, just S of Hwy 30 (JEPS)	AF229142, AF229143	
Foster s.n., Wasco Co.: Edge of The Dalles (DAV)		$n = 12$ (Cited in Foster 1972 as "The Dalles")
WASHINGTON		
* *Sandbergh 287*, Douglas Co.: Junction of Crab and Wilson Creeks (UC)		
* *Eyerdam 1232*, Mason Co.: 20 mi SW of Shelton, on shore of small lake (UC)		
* *Meyer 1551*, Thurston Co.: 4 mi W of Olympia along Hwy 101 (UC)		
* *Sandberg & Leiberg s.n.*, Eastern WA (UC)		

APPENDIX 1. CONTINUED.

Voucher	GenBank sequence	Chromosome number
Downingia bicornuta		
CALIFORNIA		
* *Schultheis 100-95*, Tehama Co.: Dale's Lake. NE of Red Bluff, on Rt A-6 (JEPS)	AF163339, AF163340, AF176867	
Downingia concolor		
CALIFORNIA		
* *Schultheis 195-95*, Napa Co.: Junction of Pope Valley Cross Rd and Chiles-Pope Valley Rd. (JEPS)	AF163363, AF176873	
Schultheis 287-95, San Diego Co.: Grown from seed collected by E. Bauder at Cuyamaca Lake site 10 (see Bauder 1992) (JEPS)	AF163396, AF163397	
Downingia cuspidata		
CALIFORNIA		
* *Schultheis 179-95*, Calaveras Co.: SE of Camanche Reservoir, at junctions of Burson Rd, Hwy 26, and Milton Rd. (JEPS)		
Schultheis 197-95, Lake Co.: Loch Lomond. Meadow, W side of Rt 175, just N of intersection with Loch Lomond Rd. (JEPS)	AF163364, AF176890	
Downingia montana		
CALIFORNIA		
* *Schultheis 235-95*, Shasta Co.: Rt 89, 3.2 mi S of Shasta/Siskiyou county line (JEPS)	AF163378, AF163379, AF176876	
Schultheis 250-95, Butte Co.: Humboldt Rd, off of Rt 32, 0.3 mi E of Butte Meadows Natl. Forest Campground (JEPS)	AF163391, AF176894	
Downingia ornatissima		
CALIFORNIA		
* *Schultheis 180-95*, Stanislaus Co.: N of Turlock Lake. S side of Barnett Rd, off of Crabtree Rd. (JEPS)	AF163360, AF176879	